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1	L3	17	spa-1	USPAT; US-PGPUB	2003/05/13 14:52
2	L4	1714	nfkb or (nf adj kappa adj b) or (nf adj kb)	USPAT; US-PGPUB	2003/05/13 14:53
3	L5	4	3 and 4	USPAT; US-PGPUB	2003/05/13 14:54

PGPUB-DOCUMENT-NUMBER: 20030087239

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030087239 A1

TITLE: Target activated nucleic acid biosensor and methods of
using same

PUBLICATION-DATE: May 8, 2003

INVENTOR-INFORMATION:

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APPL-NO: 09/ 952680

DATE FILED: September 13, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60232454 20000913 US

US-CL-CURRENT: 435/6, 536/24.3

ABSTRACT:

Methods for engineering a target activated biosensor are provided. Biosensors comprise a plurality of nucleic acid sensor molecules labeled with a first signaling moiety and a second signaling moiety. The nucleic acid sensor molecules recognizes target molecules which do not naturally bind to DNA. Binding of a target molecule to the sensor molecules triggers a change in the proximity of the signaling moieties which leads to a change in the optical properties of the nucleic acid sensor molecules on the biosensor. Reagents and systems for performing the method are also provided. The method is useful in diagnostic applications and drug optimization.

RELATED APPLICATION

[0001] This application claims priority to U.S. Ser. No. 60/232,454, filed Sep. 13, 2000. The contents of this application are incorporated herein by reference in their entirety.

----- KWIC -----

Detail Description Paragraph - DETX (202):

[0243] In one embodiment, a pathway biosensor array is generated comprising target activatable nucleic acid sensor molecules which are activatable by components of a cell cycle pathway. In this embodiment, a cell cycle biosensor is generated comprising nucleic acid nucleic acid sensor molecules activatable by at least two members selected from the group consisting of: MPS, Cytostatic factor (CSF) (including Mos), cdk4, cyclins D1-3, cdk6, cdk2, cyclin E, p53, p21, p16, Rb, p27, E2F, cyclin A, cyclin B, cdk1, cyclin B1-3, Cdc2, **SPA-1**, and other biomolecules involved in cell cycle regulation.

Detail Description Table CWU - DETL (2):

2 apoptotic pathways Bcl, Bak, ICE proteases, Ich-1, CrmA, CPP32, APO-1/Fas, DR3, FADD containing proteins, perform, p55 tumor necrosis factor (TNF) receptor, NAIP, TAP, TRADD-TRAF2 and TRADD-FADD, TNF, D4-GDI, **NF-kB**, CPP32/apopain, CD40, IRF-i, p53, apoptin blood clotting pathways thrombin, fibrinogen, factor V, Factor VIII- FVa, FVIIIa, Factor XI, Factor Xia, Factors IX and X, thrombin receptor, thrombomodulin (TM), protein C (PC) to activated protein C (aPC). aPC, plasminogen activator inhibitor-i (PAT-i), tPA (tissue plasminogen activator) calcium signaling pathways calmodulin, calcineurin, Cell cycle G0 MPS, CYTOSTATIC FACTOR (CSF) (INCLUDING MOS) Pathway G1 mid G1 phase: cdk4/cyclin D1-3 and cdk6/cyclin D1-3 late G1 phase: cdk2/cyclin E others: p53, p21, p16, Rb, p27, E2F, Cdc25A, Cdc25B S cyclin A/CDK2, cyclin B/Cdc2, SPA-i, Cdc25A, Cdc25B G2 G2/M transition phase: cdk1/cyclin B 1-3, cdk1/cyclin A, Cdc25A, Cdc25B, Cdc25C. PIN1, Chk1, Myt 1, Wee 1 M Cdc2/cyclin B, P1k, Cdc25C, Cholesterol metabolism pathway LDL, LDL-receptor, VLDL, HDL, cholesterol acyltransferase, apoprotein E, Cholesteryl esters, ApoA-I and A-II, HMGCoA reductase, cholesterol Flt-3 pathway flt-3 pathway flt-3, GRP-2, SHP-2, SHIP, Shc JAK/STATS signaling pathway Jak1, Jak2, IL-2, IL-4 and IL-7, Jak3, Ptk-2, Tyk2, EPO, GH, prolactin, IL-3, GM-CSF, G-CSF, IFN gamma, LW, OSM, IL- 12 and IL-6, IFNR-alpha, IFNR-gamma, IL-2R beta, IL-6R, CNTFR, Stat 1 alpha, Stat 1 beta, and Stats2-6 MAP kinase signaling pathways flt-3, ras, raf, Grb2, Erk-i, Erk-2, and Src, Erb2, gpl30, MEK-1, MEK-2, hsp 90, JNK, p38, Sin1, Sty1/Spcl, MKK's, MAPKAP kinase-2, TNKISAPK P53 pathway bax, bid, caspases, cytochrome c PI 3 kinase pathway SHIP, Akt ras activation pathways p120-Ras GAP, neurofibromin, Gap1, Raf-GDS, Rb1, 2, and 4, Rin1, MEKK- 1, and phosphatidylinositol-3-OH kinase (P13K), ras SIP signaling pathways GRB2, SIP, ras, P13-kinase SHC signaling pathways trkA, trkB, NGF, BDNF, NT-4/5, trkC, fNT-3, Shc, PLC gamma 1, P1-3 kinase, SNT, ras, raf, MEK and MAP kinase TGF-13 signaling pathways BMP, Smad 2, Smad4, activin, TGF T-cell receptor complex Ick, fyn, CD4, CD8, T cell receptor proteins MHC-I pathways TAP proteins, LMP 2, LMP 7, gp 96, HSP 90, HSP 70

PGPUB-DOCUMENT-NUMBER: 20030064408

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030064408 A1

TITLE: Protein-protein interactions

PUBLICATION-DATE: April 3, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 035343

DATE FILED: January 4, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60259572 20010104 US

US-CL-CURRENT: 435/7.1, 435/194 , 435/7.92 , 530/388.26

ABSTRACT:

The present invention relates to the discovery of novel protein-protein interactions that are involved in mammalian physiological pathways, including physiological disorders or diseases. Examples of physiological disorders and diseases include non-insulin dependent diabetes mellitus (NIDDM), neurodegenerative disorders, such as Alzheimer's Disease (AD), and the like. Thus, the present invention is directed to complexes of these proteins and/or their fragments, antibodies to the complexes, diagnosis of physiological generative disorders (including diagnosis of a predisposition to and diagnosis of the existence of the disorder), drug screening for agents which modulate the interaction of proteins described herein, and identification of additional proteins in the pathway common to the proteins described herein.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is related to U.S. provisional patent application Serial No. 60/259,572, filed on Jan. 4, 2001, incorporated herein by reference, and claims priority thereto under 35 USC .sctn.119(e).

----- KWIC -----

Summary of Invention Paragraph - BSTX (32):

[0030] Nuclear factor kappaB (**NFkB**) is an inducible transcription factor that regulates a large number of genes, particularly those involved in the inflammatory and immune responses (Barnes and Karin, 1997; Baeuerle and Baichwal, 1997). **NFkB** has been demonstrated to be inappropriately regulated in a number of human inflammatory disorders, including rheumatoid and osteoarthritis, asthma, arteriosclerosis and inflammatory bowel disease, as well as some cancers (Luque and Gelinas, 1997; Foxwell et al., 1998; Barnes and Adcock, 1998; Neurath et al., 1998; Hatada et al., 2000). Inhibiting **NFkB** activation has many potential applications in treating these diseases, and consequently is an area of intense interest for drug development. One mechanism by which steroids exert their broad-spectrum anti-inflammatory action is by inhibiting the activation of **NFkB**. By identifying non-steroidal means of inhibiting **NFkB** activation, it is hoped a class of novel immunosuppressive drugs that has the potency of steroids without their toxicity can be developed.

Summary of Invention Paragraph - BSTX (33):

[0031] **NFkB** activity is controlled by protein-protein interactions that alter its subcellular localization (Karin and Ben-Neriah, 2000; Karin, 1999; Mercurio and Manning, 1999). In unstimulated cells, **NFkB** is inactive and sequestered in the cytoplasm due to its interaction with I κ B, which masks the **NFkB** nuclear localization signal. Upon stimulation, I κ B is phosphorylated, which targets it for ubiquitination and proteasome-mediated degradation. Disruption of the I κ B/**NFkB** complex frees **NFkB** to enter the nucleus and activate transcription of proinflammatory genes. A key step in **NFkB** activation is the initial phosphorylation of I κ B; this is accomplished by I κ B-kinase (IKK) family members, which are in turn responsive to signals from cell surface receptors for factors such as TNF-alpha and IL-1. Clearly, identifying all of the proteins involved in **NFkB** activation is necessary to understand the process by which extracellular signals are transduced into **NFkB**-mediated transcriptional responses. Furthermore, identification of these proteins will increase the repertoire of potential targets for therapeutic intervention in the treatment of diseases due to defects involving **NFkB** activation, such as arthritis, asthma, and cancer.

Summary of Invention Paragraph - BSTX (34):

[0032] I κ B kinases (IKKs) are responsible for signal-induced phosphorylation I κ B, leading to I κ B degradation and activation of **NFkB**. These proteins appear to function as a complex of IKK family members, and may interact with other cellular factors as well. Consequently, the IKKs and proteins with which they interact are potential targets of anti-inflammatory (and other) drugs. Four IKKs [IKK-alpha (IKK α), IKK-beta (IKK β), IKK-gamma (IKK γ), and inducible IKK (IKK-i)] have been identified (reviewed in Karin and Ben-Neriah, 2000; Karin, 1999; Mercurio and Manning, 1998-10). These proteins were used in yeast two-hybrid assays to identify IKK-interacting proteins.

Summary of Invention Paragraph - BSTX (37):

[0035] The next IKKb interactor is the lactate dehydrogenase M chain (also known as LDH-A) was found to be an interactor. LDH is the last enzyme involved in anaerobic glycolysis, and resides in the cytosol. Although the significance of this interaction is not entirely clear, the demonstrated interaction with IKKb suggests that LDH can act as a phosphorylation substrate of IKKb, and further suggests a link between **NFkB** activation and cellular metabolism.

Summary of Invention Paragraph - BSTX (40):

[0038] The next interactor, the glioblastoma cell differentiation-related protein GBDR1, was found in yeast two-hybrid searches using both IKK-alpha and IKK-beta. The function of GBDR1 is not known but sequence analysis indicates the presence of two ubiquitin-associated domains. Consistent with this, the IKK-beta used to isolate GBDR1 contains a ubiquitin-like domain. In contrast, the fragment of IKK-alpha that associates with GBDR1 includes a helix-loop-helix domain rather than the ubiquitin-like domain. Nonetheless, the interaction of the same domain of GBDR1 with two different IKKs strongly suggests this protein is part of the signal transduction cascade that leads to **NFkB** activation.

Summary of Invention Paragraph - BSTX (41):

[0039] One interactor for IKK-gamma (IKKg, also known as NEMO) was identified. This protein, I-TRAF, is a known component of the **NFkB** activation cascade. I-TRAF is known to bind to the conserved C-terminal domain of TRAF proteins and inhibit TRAF-mediated **NF-kappa-B** activation (Ling and Goeddel, 2000). Phosphorylation of I-TRAF results in its dissociation from TRAF and the subsequent activation of **NFkB**. We and others have found that another IKK (IKK-i) is able to interact with, and phosphorylate, I-TRAF (Nomura et al., 2000). The interaction with IKK-gamma may similarly result in modification of I-TRAF. However, such a role for IKKg is likely indirect, since IKKg appears to be a non-catalytic IKK family member. This notion is consistent with the fact that the domain of IKK-i with which I-TRAF interacts is a C-terminal (non-kinase) region of the protein.

Summary of Invention Paragraph - BSTX (42):

[0040] The inducible Ikb kinase (IKK-i) was found to interact with three proteins. The first of these is the signal-induced proliferation associated protein SPA1. **SPA-1** is over 90% identical to the murine homolog, which was originally isolated based on its inducible expression in lymphoid cells stimulated with IL-2; it was further shown that murine SPA1 hampers mitogen-induced cell cycle progression when abnormally or prematurely expressed (Hattori et al., 1995). The N-terminal domains of both the human and murine SPA1 proteins are highly homologous to the human Rap1 GTPase-activating protein (GAP). Human SPA1 exhibits GAP activity for Rap1 and Rap2, but not for Ras, Rho, or Ran (Kurachi et al., 1997). In addition to the N-terminal GTPase activating domain, human SPA1 contains predicted coiled-coil, PDZ, and transmembrane domains. Human SPA1 is localized primarily to the perinuclear

region and is widely expressed, with highest expression levels in lymphoid organs. The interaction with IKK-i suggests SPA-1 is involved in NFkB activation.

Summary of Invention Paragraph - BSTX (43):

[0041] IKK-i is also found to interact with the nuclear mitotic apparatus protein NUMA1. NUMA1 is found in the nucleus during interphase and is associated with isolated nuclear matrices, and specifically localizes to the spindle apparatus during mitosis in a manner that suggests it is involved in the early steps of nuclear reassembly (Lydersen and Pettijohn, 1980). Analysis of the 2101 amino acid NUMA1 protein reveals an unusually long central coiled-coil domain (>1400 amino acids). Interestingly, NUMA1 is one of a handful of proteins to which RAR-alpha can be fused in acute promyelocytic leukemia (APL). The most prevalent RAR-alpha fusion partner in APL is PML, and it has been proposed that disruption of PML organization is responsible for the APL phenotype. In rare cases of APL, the ligand- and DNA-binding domains of RAR-alpha are fused to the 5' exons of NUMA1, resulting in a fusion protein that exists in sheet-like nuclear aggregates (Wells et al., 1997). Wells et al. further demonstrate that PML organization is normal in cells expressing the RAR-alpha/NUMA1 fusion, suggesting that interference with retinoid signaling, and not disruption of PML organization, is essential to the APL phenotype and implicating an element of the mitotic apparatus in the molecular pathogenesis of human malignancy. The interaction of NUMA1 with an IKK suggests that cellular processes, such as mitosis and nuclear assembly, are under control of the same signaling pathways that activate NFkB. In support of this, we have previously found interactions between NUMA1 and the signaling proteins MAPKAP-K3, PRAK, AKT1, and AKT2.

Detail Description Paragraph - DETX (44):

[0100] Baeuerle, P. and Baichwal, V. (1997). NF-kappa B as a frequent target of immunosuppressive and anti-inflammatory molecules. Adv. Immunol. 65:111-137

Detail Description Paragraph - DETX (54):

[0110] Hatada, E. N. et al. (2000). NF-KB and the innate immune response. Current Opinion in Immunology 12:52-58.

Detail Description Paragraph - DETX (56):

[0112] Karin, M. (1999). The beginning of the end: Ikb kinase (IKK) and NFkB activation. J. Biol. Chem. 274:27339-42.

Detail Description Paragraph - DETX (57):

[0113] Karin, M. and Ben-Neriah, Y. (2000). Phosphorylation meets

ubiquitination: the control of **NF-kB** Activity. Annual Review of Immunology 18:621-663.

Detail Description Paragraph - DETX (60):

[0116] Kurachi, H. et al. (1997). Human **SPA-1** gene product selectively expressed in lymphoid tissues is a specific GTPase-activating protein for Rap1 and Rap2. Segregate expression profiles from a rap1 GAP gene product. J. Biol Chem. 272:28081-8.

Detail Description Paragraph - DETX (63):

[0119] Luque, I. and Gelinas, C. (1997). Rel/**NF-kappa B** and I kappa B factors in oncogenesis. Semin. Cancer Biol. 8:103-111.

Detail Description Paragraph - DETX (65):

[0121] Mercurio, F. and Manning, A. M. (1999). Multiple signals converging on **NF-kB**. Curr. Opin Cell Biol. 11:226-32

Detail Description Table CWU - DETL (3):

15TABLE 15 Ex. BAIT ACCESSION COORDINATES PREY ACCESSION COORDINATES
4
IKKb GB: AF031416 AA: 301-602 EIF3S10 GB: D50929 AA 666-852 5 IKKb GB: AF031416 AA 301-602 SLAP2 GB: AF100750 AA 16-258 6 IKKb GB: AF031416 AA 301-602 KIAA0614 GB: AB014514 AA 549-874 7 IKKb GB: AF031416 AA 301-602 SART-1 GB:AB006198 AA 248-419 8 IKKb GB: AF031416 AA 301-602 GBDR1 GB: NM_006318 AA 4-114 9 IKKa GB: AF009225 AA 599-638 GBDR1 GB: NM_006318 AA 4-114 10 IKKg GB: AF074382 AA 150-302 I-TRAF GB: U59683 AA 17-424 11 IKK-i GB: D63485 AA 450-717 I-TRAF GB: U59683 AA 17-424 12 IKK-i GB: D63485 AA 450-717 NUMA1 GB: Z11583 AA962-1092 13 IKK-i GB: D63485 AA 450-717 **SPA-1** GB: AB005666 AA 925-1042

Claims Text - CLTX (2):

1. An isolated protein complex comprising two proteins, the protein complex selected from the group consisting of: (i) a complex of a first protein and a second protein; (ii) a complex of a fragment of said first protein and said second protein; (iii) a complex of said first protein and a fragment of said second protein; and (iv) a complex of a fragment of said first protein and a fragment of said second protein, wherein said first and second proteins are selected from the group consisting of: (a) said first protein is IKKb and said second protein is selected from the group consisting of LDHM, EIF3S10, SLAP2, KIAA0614, SART-1 and GBDR1; (b) said first protein is IKKa and said second protein is GBDR1; (c) said first protein is IKKg and said second protein is TRAF; and (d) said first protein is IKK-i and said second protein is selected from the group consisting of NUMA1, **SPA-1** and PN13730.

PGPUB-DOCUMENT-NUMBER: 20030059908

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030059908 A1

TITLE: Nucleic acids, proteins, and antibodies

PUBLICATION-DATE: March 27, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 091504

DATE FILED: March 7, 2002

RELATED-US-APPL-DATA:

child 10091504 A1 20020307

parent continuation-of 09764869 20010117 US ABANDONED

non-provisional-of-provisional 60179065 20000131 US

non-provisional-of-provisional 60180628 20000204 US

non-provisional-of-provisional 60214886 20000628 US

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non-provisional-of-provisional 60259678 20010105 US

US-CL-CURRENT: 435/183, 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

The present invention relates to novel cardiovascular system related polynucleotides and the polypeptides encoded by these polynucleotides herein collectively known as "cardiovascular system antigens," and the use of such cardiovascular system antigens for detecting disorders of the cardiovascular system, particularly the presence of cancer of cardiovascular system tissues and cancer metastases. More specifically, isolated cardiovascular system associated nucleic acid molecules are provided encoding novel cardiovascular system associated polypeptides. Novel cardiovascular system polypeptides and antibodies that bind to these polypeptides are provided. Also provided are vectors, host cells, and recombinant and synthetic methods for producing human cardiovascular system associated polynucleotides and/or polypeptides. The invention further relates to diagnostic and therapeutic methods useful for diagnosing, treating, preventing and/or prognosing disorders related to the

cardiovascular system, including cancer of cardiovascular system tissues, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of polynucleotides and polypeptides of the invention. The present invention further relates to methods and/or compositions for inhibiting the production and function of the polypeptides of the present invention.

----- KWIC -----

Summary of Invention - Table CWU - BSTL (28):

3TABLE 2 SEQ Score/ Clone ID Contig ID Analysis PFam/NR Accession Percent
 NO: Z ID: NO: X Method PFam/NR Description Number Identity NT From NT To
 HAHEE05 928673 20 blastx.2 (AC005531) similar to gb.vertline.AAD04728.1.v-
 ertline. 100% 39 422 mouse homeodomain- interacting protein 1 HAHHE12
 969107 24 blastx.14 N-RAP [Mus musculus]
 gi.vertline.2351568.vertline.gb.vertline.AAC5 76% 307 621 3323.1.vertline. 67%
 139 333 54% 460 612 78% 667 762 35% 514 708 75% 673 759 59% 481 576 32%
 526 708 37% 598 717 34% 541 705 40% 601 705 34% 547 675 27% 457 621 27%
 514 708 85% 762 803 26% 619 756 31% 541 693 55% 703 762 29% 544 675 30%
 460 585 38% 619 696 34% 598 693 38% 619 696 47% 526 588 29% 598 690 29%
 607 717 66% 457 492 47% 526 576 75% 627 662 40% 526 585 33% 616 696 36%
 649 705 23% 460 585 37% 637 708 37% 637 708 56% 526 573 39% 723 791 57%
 532 573 50% 762 797 50% 762 797 28% 711 794 27% 592 702 26% 526 594 29%
 720 800 30% 415 483 HELEA45 954371 49 blastx.14 exopolyphosphatase
 gi.vertline.147343.vertline.gb.vertline.AAA24 89% 86 169 [Escherichia coli]
 415.1.vertline. 100% 66 98 HELHF07 949067 78 HMMER PFAM: PF00202 38.85 95 295
 1.8 Aminotransferases class- III pyridoxal-phosphate blastx.14
 4-aminobutyrate gi.vertline.1742132.vertline.dbj.vertline.BAA1 85% 83 295
 aminotransferase (EC 4871.1.vertline. 92% 21 98 2.6.1.19) 11 45% 246 311
 aminotransferase). 100% 1 18 [Escherichia coli] HEMEK19 574209 92 HMMER PFAM:
 TonB dependent PF00593 46.8 81 233 2.1.1 receptor C-terminal region HEMEU54
 947801 94 blastx.14 samaphorin G [Mus gi.vertline.1418942.vertlin-
 e.emb.vertline.CAA 96% 11 325 musculus] 66398.1.vertline. 50% 122 280 42%
 128 298 51% 122 238 54% 14 112 35% 68 235 33% 134 328 42% 29 112 30% 122
 238 41% 343 429 44% 248 301 55% 302 328 62% 305 328 45% 296 328 62% 305
 328 HHBEM70 756949 107 HMMER PFAM: Core histones PF00125 13.08 144 215 1.8
 H2A, H2B, H3 and H4 HHBHO63 906947 121 HMMER PFAM: Phorbol esters/ PF00130
 2.21 188 217 1.8 diacylglycerol binding domain HHFBX77 959805 136
 blastx.14 (AB012308) B2HC gi.vertline.4033608.vertline.dbj.ver- tline.BAA3 83%
 13 366 [Anthocidaris crassispina] 5136.1.vertline. HHFCA64 720849 137 HMMER
 PFAM: Zinc-binding PF00099 3.2 287 267 1.8 metalloprotease domain HHFGN31
 908508 177 HMMER PFAM: KRAB box PF01352 64.1 93 215 2.1.1 HHFHC02 920510 192
 HMMER PFAM: Eukaryotic protein PF00069 13.11 118 183 1.8 kinase domain
 blastx.14 (AB023658) gi.vertline.4512334.vertline.dbj.vertline.BAA7 94% 70 183
 Ca/calmodulin-dependent 5246.1.vertline. protein kinase 1 HHFJN02 918358 214
 blastx.14 retrovirus-related reverse pir.vertline.B25313.vertline.GNLRL1 53%
 297 208 transcriptase pseudogene- 54% 390 286 slow loris HHFON19 910891 257
 HMMER PFAM: Dual specificity PF00782 154.8 316 732 2.1.1 phosphatase,
 catalytic domain blastx.14 (AF143321) unknown
 gi.vertline.4929222.vertline.gb.vertline.AAD3 68% 298 825 [Homo sapiens]

3910.1.vertline.AF143321_1 HHFUC26 960331 267 HMMER PFAM: Src homology
 PF00018 3.21 343 375 1.8 domain 3 HMEGH46 887791 287 HMMER PFAM: C2 domain
 PF00168 12.81 10 78 1.8 HULAI37 708923 307 HMMER PFAM: Core histones PF00125
 13.48 99 173 1.8 H2A, H2B, H3 and H4 HULFB76 767873 313 HMMER PFAM: HIT
 family PF01230 24.6 67 147 2.1.1 HUSIW10 963324 330 blastx.14 (AF098499) No
 definition gi.vertline.3786408.vertlin- e.gb.vertline.AAC6 48% 234 320 line
 found [Caenorhabditis 7396.1.vertline. 41% 149 241 elegans] HUSYA63 928021
 335 blastx.14 (AF116865) hedgehog- gi.vertline.4868122.vertline.gb.vertli-
 ne.AAD3 88% 251 439 interacting protein [Mus 1172.1.vertline.AF116865_1
 musculus] HUSZH03 922852 341 blastx.14 C06A6.3 gene product
 gi.vertline.1086626.vertline.gb.vertline.A- AA8 34% 367 633 [Caenorhabditis
 elegans] 2295.1.vertline. 61% 259 297 HUSYN11 943237 345 HMMER PFAM: Core
 histones PF00125 13.67 238 315 1.8 H2A, H2B, H3 and H4 blastx.2 (AL137556)
 hypothetical emb.vertline.CAB70810.1.vertline. 67% 137 319 protein [Homo
 sapiens] 96% 240 320 96% 241 321 HUSIE95 967176 366 blastx.14 GS2NA [Homo
 sapiens] gi.vertline.805095.vert- line.gb.vertline.AAB81 56% 229 5
 551.1.vertline. 53% 496 413 37% 121 11 31% 388 332 33% 484 431 HUSIE08
 908574 368 blastx.14 (AB024005) KRAB-
 gi.vertline.4514561.vertline.dbj.vertline.BAA7 70% 38 229 containing
 zinc-finger 5468.1.vertline. protein KRAZ2 [Mus musculus] HUSHL86 960355 369
 blastx.14 (AF151805) CGI-47 gi.vertline.4929563.vertline.gb.vertline.AAD3 96%
 1142 882 protein [Homo sapiens] 4042.1.vertline.AF151805_1 100% 1413 1330
 HUSFF03 924616 389 blastx.14 (AF033276) A kinase gi.vertline.2852701.vert-
 line.gb.vertline.AAC0 83% 266 535 anchor protein [Mus 2208.1.vertline. 47%
 541 591 musculus] HHFLU06 857884 445 HMMER PFAM: Adenylate and PF00211 108.8
 17 268 2.1.1 Guanylate cyclase catalytic domain HHFKX28 971102 453
 blastx.14 Similarity to Yeast gi.vertline.3881836.vertline.emb.vertline.C- AB
 76% 858 619 LPG22P protein 01454.1.vertline. 65% 495 409 (TR: G1151240); 11
 91% 617 546 cDNA EST EMBL: C10626 comes from this gene; cDNA EST EMBL:
 C10848 HHFJM64 958384 455 blastx.2 (AF026504) **SPA-1** like
 gb.vertline.AAB81526.1.vertline. 83% 3 287 protein p1294 [Rattus 43% 323 664
 norvegicus] 29% 799 1266 28% 847 1344 HHFCH52 911570 503 blastx.14 INSERTIN =
 TENSIN sp.vertline.G256713.vertline.G256713 95% 15 77 HOMOLOG. HHBGJ53
 909912 525 HMMER PFAM: PH domain PF00169 38.3 160 267 2.1.1 HHBGG10 963849
 526 blastx.14 (AB011527) MEGF1 gi.vertline.3449286.vertline.dbj.vertline.BAA3
 90% 98 3 [Rattus norvegicus] 2458.1.vertline. 75% 210 112 45% 210 151 41%
 219 184 HHBEG80 951688 533 HMMER PFAM: Core histones PF00125 12.4 371 436
 1.8 H2A, H2B, H3 and H4 HEMGL56 767669 538 HMMER PFAM: Filamin/ABP280 PF00630
 84.1 45 209 2.1.1 repeat. HEMDX96 935963 543 blastx.14 (AF111170) unknown
 gi.vertline.4314286.vertline.gb.vertline.AAD1 79% 491 255 [Homo sapiens]
 5563.1.vertline. HEMBT61 939957 550 HMMER PFAM: Eukaryotic protein PF00069
 76.6 16 285 2.1.1 kinase domain blastx.2 (AD000092) hypothetical
 gb.vertline.AAB51171.1.vertline. 71% 13 441 human serine-threonine protein
 kinase R31240_1 [Homo sapiens] HELGY02 948302 557 blastx.2 Similar to
 sulfatase gb.vertline.AAA83618.1.vertline. 59% 383 523 [Caenorhabditis
 elegans] HELGW31 610003 558 HMMER PFAM: Cytochrome C PF01578 216.5 672 1286
 2.1.1 assembly protein blastx.2 (AE000309) heme
 gb.vertline.AAC75259.1.vertline. 100% 603 1337 exporter protein C
 [Escherichia coli] HELGW31 957568 622 HMMER PFAM: Cytochrome C PF01578 200.9
 990 421 2.1.1 assembly protein blastx.2 (AE000309) heme
 gb.vertline.AAC75259.1.vertline. 99% 5 619 exporter protein C 100% 621 713
 [Escherichia coli] HELGW31 964303 623 blastx.2 yejV [Escherichia coli]
 gb.vertline.AAA16392.1.vertline. 93% 39 449 60% 1 75 HELGK56 925698 561

HMMER PFAM: Pyruvate

Detail Description Paragraph - DETX (374):

[1283] Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 34 and 35. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/**NF-KB**/EGR, GAS/**NF-KB**, I1-2/NFAT, or **NF-KB**/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

Detail Description Paragraph - DETX (411):

[1316] **NF-KB** (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, **NF-KB** regulates the expression of genes involved in immune cell activation, control of apoptosis (**NF-KB** appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

Detail Description Paragraph - DETX (412):

[1317] In non-stimulated conditions, **NF-KB** is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I-KB is phosphorylated and degraded, causing **NF-KB** to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by **NF-KB** include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

Detail Description Paragraph - DETX (413):

[1318] Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the **NF-KB** promoter element are used to screen the supernatants produced in Example 30. Activators or inhibitors of **NF-KB** would be useful in treating, preventing, and/or diagnosing diseases. For example, inhibitors of **NF-KB** could be used to treat those diseases related to the acute or chronic activation of **NF-KB**, such as rheumatoid arthritis.

Detail Description Paragraph - DETX (414):

[1319] To construct a vector containing the **NF-KB** promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of

the **NF-KB** binding site (GGGGACTTTCCC) (SEQ ID NO: 8), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

Detail Description Paragraph - DETX (418):

[1323] Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this **NF-KB**/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Detail Description Paragraph - DETX (419):

[1324] In order to generate stable mammalian cell lines, the **NF-KB**/SV40/SEAP cassette is removed from the above **NF-KB**/SEAP vector using restriction enzymes Sall and NotI, and inserted into a vector containing neomycin resistance. Particularly, the **NF-KB**/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with Sall and NotI.

Detail Description Paragraph - DETX (420):

[1325] Once **NF-KB**/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 32. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 32. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

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INVENTOR-INFORMATION:

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APPL-NO: 09/ 764869

DATE FILED: January 17, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60179065 20000131 US

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ABSTRACT:

The present invention relates to novel cardiovascular system related polynucleotides and the polypeptides encoded by these polynucleotides herein collectively known as "cardiovascular system antigens," and the use of such cardiovascular system antigens for detecting disorders of the cardiovascular system, particularly the presence of cancer of cardiovascular system tissues and cancer metastases. More specifically, isolated cardiovascular system associated nucleic acid molecules are provided encoding novel cardiovascular system associated polypeptides. Novel cardiovascular system polypeptides and antibodies that bind to these polypeptides are provided. Also provided are vectors, host cells, and recombinant and synthetic methods for producing human cardiovascular system associated polynucleotides and/or polypeptides. The invention further relates to diagnostic and therapeutic methods useful for diagnosing, treating, preventing and/or prognosing disorders related to the cardiovascular system, including cancer of cardiovascular system tissues, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of polynucleotides and polypeptides of the invention. The present invention further relates to methods and/or compositions for inhibiting the production and function of the polypeptides of the present invention.

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Summary of Invention - Table CWU - BSTL (30):

4TABLE 2 SEQ Score/ Clone ID Contig ID Analysis PFam/NR Accession Percent
 NO : Z ID: NO : X Method PFam/NR Description Number Identity NT From NT To
 HAHEE05 928673 20 blastx.2 (AC005531) similar to gb.vertline.AAD04728.1.v-
 ertline. 100% 39 422 mouse homeodomain- interacting protein 1 HAHHE12
 969107 24 blastx.14 N-RAP [Mus musculus]
 gi.vertline.2351568.vertline.gb.vertline.AAC5 76% 307 621 3323.1.vertline. 67%
 139 333 54% 460 612 78% 667 762 35% 514 708 75% 673 759 59% 481 576 32%
 526 708 37% 598 717 34% 541 705 40% 601 705 34% 547 675 27% 457 621 27%
 514 708 85% 762 803 26% 619 756 31% 541 693 55% 703 769 29% 544 675 30%
 460 585 38% 619 696 34% 598 693 38% 619 696 47% 526 588 29% 598 690 29%
 607 717 66% 457 492 47% 526 576 75% 627 662 40% 526 585 33% 616 696 36%
 649 705 23% 460 585 37% 637 708 37% 637 708 56% 526 573 39% 723 791 57%
 532 573 50% 762 797 50% 762 797 28% 711 794 27% 592 702 26% 526 594 29%
 720 800 30% 415 483 HELEA45 954371 49 blastx.14 exopolyphosphatase
 gi.vertline.147343.vertline.gb.vertline.AAA24 89% 86 169 [Escherichia coli]
 415.1.vertline. 100% 66 98 HELHF07 949067 78 HMMER PFAM: PF00202 38.85 95 295
 1.8 Aminotransferases class- III pyridoxal-phosphate blastx.14
 4-aminobutyrate gi.vertline.1742132.vertline.dbj.vertline.BAA1 85% 83 295
 aminotransferase (EC 4871.1.vertline. 92% 21 98 2.6.1.19) 1 1 45% 246 311
 aminotransferase). 100% 1 18 [Escherichia coli] HEMEK19 574209 92 HMMER PFAM:
 TonB dependent PF00593 46.8 81 233 2.1.1 receptor C-terminal region HEMEU54
 947801 94 blastx.14 samaphorin G [Mus gi.vertline.1418942.vertlin-
 e.emb.vertline.CAA 96% 11 325 musculus] 66398.1.vertline. 50% 122 280 42%
 128 298 51% 122 238 54% 14 112 35% 68 235 33% 134 328 42% 29 112 30% 122
 238 41% 343 429 44% 248 301 55% 302 328 62% 305 328 45% 296 328 62% 305
 328 HHBEM70 756949 107 HMMER PFAM: Core histones PF00125 13.08 144 215 1.8
 H2A, H2B, H3 and H4 HHBHO63 906947 121 HMMER PFAM: Phorbol esters/ PF00130
 2.21 188 217 1.8 diacylglycerol binding domain HHFBX77 959805 136
 blastx.14 (AB012308) B2HC gi.vertline.4033608.vertline.dbj.ver- tline.BAA3 83%
 13 366 [Anthocidaris crassispina] 5136.1.vertline. HHFCA64 720849 137 HMMER
 PFAM: Zinc-binding PF00099 3.2 287 267 1.8 metalloprotease domain HHFGN31
 908508 177 HMMER PFAM:KRAB box PF01352 64.1 93 215 2.1.1 HHFHC02 920510 192
 HMMER PFAM: Eukaryotic protein PF00069 13.11 118 183 1.8 kinase domain
 blastx.14 (AB023658) gi.vertline.4512334.vertline.dbj.vertline.BAA7 94% 70 183
 Ca/calmodulin-dependent 5246.1.vertline. protein kinase 1 HHFJN02 918358 214
 blastx.14 retrovirus-related reverse
 pir.vertline.B25313.vertline.GNLRL1.vertline. 53% 297 208 transcriptase
 pseudogene - 54% 390 286 slow loris HHFON19 910891 257 HMMER PFAM: Dual
 specificity PF00782 154.8 316 732 2.1.1 phosphatase, catalytic domain
 blastx.14 (AF143321) unknown gi.vertline.4929222.vertline.gb.vertline.AAD3 68%
 298 825 [Homo sapiens] 3910.1.vertline.AF143321_1 HHFUC26 960331 267 HMMER
 PFAM: Src homology PF00018 3.21 343 375 1.8 domain 3 HMEGH46 887791 287
 HMMER PFAM: C2 domain PF00168 12.81 10 78 1.8 HULAI37 708923 307 HMMER PFAM:
 Core histones PF00125 13.48 99 173 1.8 H2A, H2B, H3 and H4 HULFB76 767873
 313 HMMER PFAM: HIT family PF01230 24.6 67 147 2.1.1 HUSIW10 963324 330
 blastx.14 (AF098499) No definition
 gi.vertline.3786408.vertline.gb.vertline.AAC6 48% 234 320 line found
 [Caenorhabditis 7396.1.vertline. 41% 149 241 elegans] HUSYA63 928021 335
 blastx.14 (AF116865) hedgehog- gi.vertline.4868122.vertline.gb.vertline.AAD3
 88% 251 439 interacting protein [Mus 1172.1.vertline.AF116865_1 musculus]

HUSZH03 922852 341 blastx.14 C06A6.3 gene product
gi.vertline.1086626.vertline.gb.vertline.AAA8 34% 367 633 [Caenorhabditis
elegans] 2295.1.vertline. 61% 259 297 HUSYN11 943237 345 HMMER PFAM: Core
histones PF00125 13.67 238 315 1.8 H2A, H2B, H3 and H4 blastx.2 (AL137556)
hypothetical emb.vertline.CAB7O810.1.vertline. 67% 137 319 protein [Homo
sapiens] 96% 240 320 96% 241 321 HUSIE95 967176 366 blastx.14 GS2NA [Homo
sapiens] gi.vertline.805095.vertline.gb.vertline.AA- B81 56% 229 5
551.1.vertline. 53% 496 413 37% 121 11 31% 388 332 33% 484 431 HUSIE08
908574 368 blastx.14 (AB024005) KRAB- gi.vertline.4514561.vertline.dbj.ve-
rtline.BAA7 70% 38 229 containing zinc-finger 5468.1.vertline. protein KRAZ2
[Mus musculus] HUSHL86 960355 369 blastx.14 (AF151805) CGI-47
gi.vertline.4929563.vertline.gb.vertline.- AAD3 96% 1142 882 protein [Homo
sapiens] 4042.1.vertline.AF151805_1 100% 1413 1330 HUSFF03 924616 389
blastx.14 (AF033276) A kinase gi.vertline.2852701.vertline.gb.vertline.AA- C0
83% 266 535 anchor protein [Mus 2208.1.vertline. 47% 541 591 musculus]
HHFLU06 857884 445 HMMER PFAM: Adenylate and PF00211 108.8 17 268 2.1.1
Guanylate cyclase catalytic domain HHFKX28 971102 453 blastx.14 Similarity to
Yeast gi.vertline.3881836.vertline.emb.vertline.CAB 76% 858 619 LPG22P
protein 01454.1.vertline. 65% 495 409 (TR:G1151240); 1 1 91% 617 546 cDNA
EST EMBL:C10626 comes from this gene; cDNA EST EMBL:C10848 HHFJM64 958384
455 blastx.2 (AF026504) **SPA-1** like gb.vertline.AAB81526.1.vertline. 83% 3 287
protein p1294 [Rattus 43% 323 664 norvegicus] 29% 799 1266 28% 847 1344
HHFCH52 911570 503 blastx.14 INSERTIN=TENSIN
sp.vertline.G256713.vertline.G256713 95% 15 77 HOMOLOG. HHBGJ53 909912 525
HMMER PFAM: PH domain PF00169 38.3 160 267 2.1.1 HHBGG10 963849 526
blastx.14 (AB011527) MEGF1 gi.vertline.3449286.vertline.dbj.vertline.BAA3 90%
98 3 [Rattus norvegicus] 2458.1.vertline. 75% 210 112 45% 210 151 41% 219
184 HHBEG80 951688 533 HMMER PFAM: Core histones PF00125 12.4 371 436 1.8
H2A, H2B, H3 and H4 HEMGL56 767669 538 HMMER PFAM: Filamin/ABP280 PF00630
84.1 45 209 2.1.1 repeat. HEMDX96 935963 543 blastx.14 (AF111170) unknown
gi.vertline.4314286.vertline.gb.vertline.AAD1 79% 491 255 [Homo sapiens]
5563.1.vertline. HEMBT61 939957 550 HMMER PFAM: Eukaryotic protein PF00069
76.6 16 285 2.1.1 kinase domain blastx.2 (AD000092) hypothetical
gb.vertline.AAB51171.1.vertline. 71% 13 441 human serine-threonine protein
kinase R31240_1 [Homo sapiens] HELGY02 948302 557 blastx.2 Similar to
sulfatase gb.vertline.AAA83618.1.vertline. 59% 383 523 [Caenorhabditis
elegans] HELGW31 610003 558 HMMER PFAM: Cytochrome C PF01578 216.5 672 1286
2.1.1 assembly protein blastx.2 (AE000309) heme
gb.vertline.AAC75259.1.vertline. 100% 603 1337 exporter protein C
[Escherichia coli] HELGW31 957568 622 HMMER PFAM: Cytochrome C PF01578 200.9
990 421 2.1.1 assembly protein

Detail Description Paragraph - DETX (365):

[1235] Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 34 and 35. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/**NF-KB**/EGR, GAS/**NF-KB**, I1-2/NFAT, or **NF-KB**/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA

(epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

Detail Description Paragraph - DETX (402):

[1264] **NF-KB** (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, **NF-KB** regulates the expression of genes involved in immune cell activation, control of apoptosis (**NF-KB** appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

Detail Description Paragraph - DETX (403):

[1265] In non-stimulated conditions, **NF-KB** is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I-KB is phosphorylated and degraded, causing **NF-KB** to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by **NF-KB** include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

Detail Description Paragraph - DETX (404):

[1266] Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the **NF-KB** promoter element are used to screen the supernatants produced in Example 30. Activators or inhibitors of **NF-KB** would be useful in treating, preventing, and/or diagnosing diseases. For example, inhibitors of **NF-KB** could be used to treat those diseases related to the acute or chronic activation of **NF-KB**, such as rheumatoid arthritis.

Detail Description Paragraph - DETX (405):

[1267] To construct a vector containing the **NF-KB** promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the **NF-KB** binding site (GGGGACTTTCCC) (SEQ ID NO: 8), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

Detail Description Paragraph - DETX (408):

[1270] Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this **NF-KB**/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Detail Description Paragraph - DETX (409):

[1271] In order to generate stable mammalian cell lines, the **NF-KB**/SV40/SEAP cassette is removed from the above **NF-KB**/SEAP vector using restriction enzymes Sall and NotI, and inserted into a vector containing neomycin resistance. Particularly, the **NF-KB**/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with Sall and NotI.

Detail Description Paragraph - DETX (410):

[1272] Once **NF-KB**/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 32. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 32. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.